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Inbred strains of rats differ in their susceptibility to both spontaneous and chemically-induced mammary carcinoma formation. We are currently using molecular biology techniques combined with classical genetic breeding studies to try to identify the mammary carcinoma suppressor (MCS) gene(s) responsible for the mammary carcinoma resistance trait in inbred Copenhagen (Cop) and Wistar Kyoto (WKy) rats. Simple sequence repeat (SSR) markers from the literature as well as from our chromosome specific libraries were used to map the rat genome and fine-map the regions that involved in the resistance phenotype. Previous data identified a quantitative trait locus (QTL) on rat chromosome 2 which is linked to the resistance phenotype (the gene is termed *Mcs-1*), and a marker on rat chromosome 7 which also appeared linked to the resistance phenotype (the gene is termed *Mcs-2*). Since the last progress report, 27 additional markers have been placed on the chromosome 2 map. One marker, CA2.F1, increased the LOD score to 4.154, further confirming the QTL at *Mcs-1*. Thirteen additional markers have been placed on the chromosome 7 map, and *Mcs-2* is localized near a region on chromosome 7q13. Moreover, we found two other regions which show suggestive linkage to the resistance phenotype, one each on chromosomes 1 and 7.

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INTRODUCTION

It has been known since the 1950's that different strains of laboratory rats exhibit different rates of formation of various kinds of tumors (Dunning and Curtis, 1945). The Wistar Furth (WF) and Fisher 344 (F344) strains are highly susceptible to both spontaneous and chemically-induced mammary tumors (Gould, 1986). The Copenhagen (Cop) and Wistar Kyoto (WKy) strains are very resistant to both types of mammary cancer (Dunning and Curtis, 1945; Haag *et al.*, 1992). The resistance to mammary carcinomas in the Copenhagen rat was initially described in the 1940's. Using the chemical carcinogen 2-acetylaminoflourene (AAF), it was shown that Cop rats rarely developed mammary cancers but were not protected from the formation of hepatic cancers. Thus the cancer resistant phenotype of these rats was believed to be mammary specific (Dunning and Curtis, 1945). Research completed within the last ten years by both this laboratory and that of Dr. John Isaacs pinpointed the nature and site of action of the genes involved in this phenotype. Using classical genetic breeding studies and transplantation studies it was demonstrated that the mammary carcinoma resistance phenotype of the Cop rat was likely due to the action of a single autosomal dominant gene whose site of action lies within the mammary epithelium (Isaacs, 1986; Gould *et al., 1989*; Haag *et al.*, 1992).

The goal of this research is to map and eventually clone the rat gene(s) we term MCS (mammary carcinoma suppressor) which are responsible for the tumor resistance phenotype in the hope it will prove useful as a diagnostic indicator of breast cancer risk in humans and possibly lead to the formation of new drugs for the treatment of human breast cancers. To this end much research has already been

completed. Using four strains of rats, two which are susceptible to chemically induced mammary carcinogenesis (WF and F344) and two which are resistant (Cop and WKy), two separate backcross sets of animals were generated and tested for the formation of mammary tumors following administration of 7,12-dimethybenz (a) anthracene (DMBA). For each cross, (WKy x F344)F1 x F344 and (Cop x WF)F1 x WF, approximately 200 female offspring were generated and phenotyped. Using the relatively new techniques of mapping with mini- and microsatellite markers (Jeffreys *et al.*, 1985; Hilbert *et al.*, 1991; Jacob *et al.*, 1991) a linkage map was created for the two backcross sets. Briefly mini- and microsatellite markers are composed of simple sequence repeats (SSR) 1-60 base pairs in length and often contain dinucleotide repeats (Miesfeld *et al.*, 1981; Hamada *et al.*, 1982; Stallings *et al.*, 1991). The length of repeats can vary from strain to strain thus making them useful for genetic mapping (Weber, 1990a, b). Microsatellite markers are distinguished on sequencing gels following a polymerase chain reaction (PCR) using primers located on either side of the repeat, while minisatellite markers are resolved on genomic Southern blots. Genetic mapping of the MCS phenotype using these SSR markers and our two sets of backcross animals was performed using tumor number as a quantitative trait in order to allow for the possibility that resistance could be due to more than one gene locus (Lander *et al.*, 1987). Using this analysis, the minisatellite marker M13 was shown to be linked to the MCS phenotype. M13 yields a LOD score of 3.8 using quantitative analysis. It is suggested that for mapping mouse or rat loci underlying complex traits in a backcross mapping panel a LOD score threshold of 1.9 should be reported as "suggestive linkage" while that over 3.3 should be treated as "significant linkage" (Lander and Kruglyak, 1995). Fluorescence *in situ* hybridization (FISH) to whole rat chromosomes using a P1 clone containing the

sequence of marker *Mit-R1025*, which maps near M13, confirmed mapping to the centromeric end of rat chromosome 2 (Hsu *et al.*, 1994). In the last annual report we mentioned that, despite the problems with background contamination, we obtained two additional markers on chromosome 2 from a chromosome-2-specific library. These markers were designated UW5 and UW9, but neither were linked to the MCS phenotype. Additional SSR markers for chromosome 2 and the whole genome were obtained from Research Genetics, and from Dr. Howard Jacob (Medical College of Wisconsin). Of these, 8 markers were added to the map. Thus, as reported in the first progress report, along with the 2 markers from our libraries, we had added 10 markers to the rat map. Notably, marker R696 mapped 0.6 cM proximal to M13 and was linked to *Mcs-1*. This served as confirmation of the linkage of M13 to *Mcs-1*. However, more markers were still needed in this region to accurately locate *Mcs-1*.

While searching the whole rat genome, several markers on chromosome 7 appeared to be linked to the resistant phenotype. Using quantitative analysis, marker R5141 yielded a LOD score of 2.2, and R3543 yielded a LOD of 2.6. These LOD scores are suggestive of a QTL (*Mcs*-2) and the region was investigated further.

The genes discussed in this report are believed to represent new tumor suppressor genes since the known tumor suppressor gene *RB1* maps to rat chromosome 15 (Szpirer *et al.*, 1991), *p53* (Thompson *et al.*, 1990; Lindblom *et al.*, 1993) maps to rat chromosome 10 (Yasue *et al.*, 1992, Yeung *et al.*, 1993), and *BRCA1* (Hall *et al.*, 1990) also maps to rat chromosome 10 (Chen *et al.*, 1996, in press).

The main objectives of this proposed research are as follows:

1. Test additional markers over the thus far untested regions of the rat genome for linkage to the MCS phenotype (to 5 cM resolution). Confirm mapping of additional

genes to specific chromosomes by FISH to whole chromosomes using specific SSR-positive P1 clones.

2. Construct chromosome-2-specific libraries from flow-sorted chromosomes and isolate new SSR markers to fine-map (to within 1 cM resolution) the region of chromosome 2 surrounding the *Mcs-1* locus.

3. Construct chromosome-7-specific libraries from flow-sorted chromosomes and isolate new SSR markers to fine-map (to within 1 cM resolution) the region of chromosome 7 surrounding the *Mcs-2* locus.

4. Test for loss of heterozygosity of MCS-linked SSR markers in mammary tumors from two independent F1 hybrids following radiation and DMBA tumor induction.

The past year has been spent constructing new chromosome-specific libraries and trying to obtain additional SSR markers for chromosomes 2 and 7, screening the entire rat genome for linkage to the MCS phenotype with new SSR markers, and testing for loss of heterozygosity of new SSR markers.

Some objectives presented in the initial proposal were abandoned in the last year. As mentioned in the last annual report, the GDRDA (Genetically Driven Representational Difference Analysis) technique didn't provide useful markers. Therefore we abandoned this procedure and concentrated on the SSR markers. Also, because the libraries from microdissected chromosomes contained very few unique clones we stopped isolating SSR markers by this approach.

It should be noted at this point that all of the data reported in this document were a result of a larger project group of investigators. Dr. Lan (successor of Dr. Virginia Ford) focused his efforts over the last year generating and mapping new SSR markers from chromosome-7-specific libraries, and his work is therefore focused on

mapping *Mcs*-2. While Dr. Lan did not specifically conduct the work on chromosome 2 and the LOH studies, he was involved on a weekly basis with the progress and design of these experiments. As with most large mapping projects, this one is a team effort.

BODY

MATERIALS AND METHODS

Generation of Chromosome-specific Libraries

For whole chromosome 2 and chromosome 7 libraries, the DNA sources were obtained by flow cytometric sortings of rat chromosome 2 and chromosome 7 (Shepel *et al*, 1994), and the chromosomes were used directly for PCR without DNA purification.

PCR was carried out using the degenerate primer 5'CCGACTCGAGNNNNNNATGTGG3' (Telenius *et al*, 1992). Template for each reaction consisted of 300-400 copies of whole chromosome 2 or chromosome 7, and 100 pg (~30 genome equivalents) of Copenhagen DNA for a positive control. A negative control reaction containing no DNA was also performed to ensure no background contamination. Final concentrations of reagents were 10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 mM dNTPs, 1.5 mM primer, 1.25 Units Taq LD polymerase (Perkin-Elmer Cetus) in a final volume of 25 µl. Note also that for sorted chromosomes, there will be additional components in the reaction which come from the sheath fluid in which the chromosomes are sorted (some salts and spermine and spermidine), but these did not seem to inhibit the reaction. We followed the protocol of Guan *et al.* (1992) with the following cycle conditions. The reaction mixtures were first heated to 94°C for 4 min, followed by one cycle at 94°C for 1 min, 30°C annealing for 3 min, then 9 cycles at 94°C for 1 min, 30°C for 1 min, and 20 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min. The last 72°C

synthesis step was carried out for 10 min. A second round of PCR was carried out using 2.5 µl aliquots of the first round reaction products in a final volume of 50 µl per reaction. The reaction components were the same as before except that the primer now has a $(CUA)_4$ tail at the 5' end to allow easy cloning into the pAMP10 vector. Cycle conditions for the second round PCR consisted of 94°C for 5 min, 10 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min, then a final extension at 72°C for 10 min. All or half of each PCR reaction was then used directly for hybridization-selection/affinity capture as described below.

In order to isolate microsatellite markers more effectively, some modifications have been made to the method we presented in the initial proposal for cloning the DOP-PCR products. Before cloning, the DOP-PCR products were enriched for $(CA)_n$ or other simple sequence repeats by hybridization-selection followed by affinity capture on Affinitips™ (a pipette tip micro-column for streptavidin capture of biotinylated molecules; Genosys, Cat No. ASM-010). In this procedure, excessive biotin-labeled $(CA)_n$ probe (200 pmol) was hybridized with 20 µl denatured DOP-PCR products in 50 µl phosphate-SDS solution (0.5 M $NaPO_4$, pH7.4, 0.5 % SDS) in an eppendorf tube at 50°C for 2 hrs. The PCR products that contain $(CA)_n$ repeats anneal to the biotinylated probe. Then the hybridization mix was applied to the Affinitip column and the PCR products were bound and washed according to manufacturer's specifications. The single-stranded target fragments containing $(CA)_n$ repeats were eluted by H_2O at 65°C, then precipitated with ethanol and resuspended in 5 µl H_2O. This step enriches the library for $(CA)_n$-containing clones by about 150 fold. We found that up to 80% clones in the final library are positive for $(CA)_n$ repeats after this hybridization selection.

To prepare the $(CA)_n$-enriched products for insertion into the pAMP10 vector (Gibco BRL Life Technologies, Gaithersburg, MD), the eluted DNA was reamplified with the DOP-PCR primer bearing a $(CUA)_4$ tail at the 5' end. For a 20 µl reaction volume the mixture was heated to 94°C for 5 min followed by 15 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min. The final synthesis step was extended to 10 min. Two µl of this product were annealed into the pAMP10 vector (20 µl reaction volume) according to the manufacturer's directions (CloneAmp Systems, Gibco BRL). One µl of the annealed reaction was used to transform DH5αF' library efficiency competent cells (Gibco-BRL Life Technologies, Gaithersburg, MD) yielding 50-400 transformants grown under ampicillin selection.

Screening and sequencing of Chromosome-specific libraries.

Transformants containing chromosome 2- or 7-specific sequences were further screened for $(CA)_n$ repeats by a simple PCR-based method we termed Colony PCR. Briefly each clone was subjected to two 10 µl PCR reactions: one with M13 forward and reverse primers to estimate the insert size, the other with M13 forward and reverse primers plus an additional $N(CA)_{10}$ primer to determine whether there is $(CA)_n$ repeat and how far the repeat is away from the M13 primer. In the M13 forward + reverse + $N(CA)_{10}$ PCR, the $N(CA)_{10}$ primer anneals to $(CA)_n$ repeats in the insert sequence and hence gives a PCR product with either the M13 forward or reverse primer. The random base at the 5' end of the $N(CA)_{10}$ primer serves to anchor the primer at the end of the repeat sequence in order to avoid multiple priming along the repeats. Each reaction contains 10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 mM dNTPs, 2.0 µM each primer, 0.25 Units Taq

polymerase (Perkin-Elmer Cetus). The mixture was heated to 94°C for 5 min followed by 40 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 30 sec. The final synthesis step was extended to 7 min. For screening many clones the PCR can be set up in 96-well plates using a multichannel Rainin motorized pipette, overlaid with light mineral oil (Sigma) and run on an MJ Research PTC-100 Programable Thermal Cycler. The PCR products are run on a 2% agarose gel and clones that show an extra, clear band in the M13 forward + reverse + $N(CA)_{10}$ PCR are positive clones that contain $(CA)_n$ repeats.

Positive clones were isolated and plasmid DNA for sequencing was extracted by alkaline-lysis method using the QIAGEN plasmid purification system (QIAGEN Inc, California). DNA sequencing to identify the unique DNA sequences surrounding the $(CA)_n$ repeats was performed using the PRISM Dye-Terminator fluorescent sequencing system (Applied Biosystems Incorporated-Perkin Elmer), and was performed at the University of Wisconsin Biotechnology Center. The insert sequence data were analyzed by computer programs GCG Version 8.2 (University of Wisconsin-Madison), or by DNAStar (DNASTAR Inc, Madison, WI) and Oligo 5.0 (National Biosciences Inc., Plymouth, MN). New primers were generated spanning the repeats and were analyzed in the four rat strains to determine which markers are informative (ie., polymorphic) in our parental strains. To measure the length variation of the SSR, genomic DNA was used as a template for PCR and PCR products are first run on an agarose gel. For markers in which strain variations were too small to be detected by agarose gel, radiolabeled deoxynucleotide was incorporated during the PCR reaction so that the product can be visualized by autoradiography or phosphorimaging (Molecular Dynamics) after electrophoresis

on a sequencing gel. Informative markers were then used to score the backcross animals for linkage to the MCS phenotype.

Linkage analysis of SSR markers

Genomic DNA samples were prepared from either rat tails or spleens of the backcross and parental animals using the standard procedure (Ausubel *et al.*, 1987). SSR marker primers were synthesized by Research Genetics (Huntsville, AL). PCR reactions were performed with [α-^{32}P]dATP (3000 Ci/mmole) and electrophoresed on 5% polyacrylamide sequencing gels. For each reaction 50 ng of genomic DNA was amplified in a 5 µl reaction containing 10 mM Tris-HCl, pH 8.3 , 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% w/V gelatin, 120 nM of each primer, 200 mM [α-^{32}P]dATP, and AmpliTaq polymerase (0.5-1 U/100 microliters, Perkin Elmer Cetus). The reactions were set up in 96-well plates using a Biomek workstation (Beckman Instruments), overlaid with mineral oil (Sigma) and run on an MJ Research Programable Thermal Cycler PTC-100 using the following cycling conditions: denaturation at 94°C for 3 min followed by 25 cycles of 95°C, 1 min; 55°C, 1 min; and 72°C, 30 sec. A final 72°C extension step was carried out for 5 min. Wet gels were transferred to Whatman 3MM paper, wrapped in plastic wrap, exposed to either film or a phosphorimager screen (Molecular Dynamics) followed by analysis by eye or the Phosphorimager (Molecular Dynamics) respectively. Phenotypes of backcross animals were subjected to linkage analysis using the MAPMAKER computer program (Lander *et al.*, 1987) and a quantitative analysis was performed with the program MAPMAKER-QTL (Lander and Botstein, 1989). In the quantitative analysis, the square root of the tumor number is dealt with as a quantitative trait and estimates the contribution of

a given locus to the phenotype, as the square roots better show parametric distribution than the tumor numbers do. Alternatively, the NEWQTL program, a newly developed nonparametric approach for mapping quantitative trait loci, was also used (Kruglyak and Lander, 1995).

Test for loss of heterozygosity of MCS-linked SSR markers

In order to identify and compare the genetic lesions associated with tumorigenesis in rats carrying *Mcs-1*, we induced mammary carcinomas in (WF X Cop)F1 rats using either DMBA or radiation. The tumors were screened for allelic imbalance using PCR and 65 polymorphic microsatellite markers spanning the genome.

DNA was isolated from tumors by proteinase K digestion and phenol:chloroform extractions. For each DNA sample, the total PCR volume was 5 µl, consisting of 2.5 µl of 20 ng/µl DNA and 2.5 µl of PCR master mix. Final concentration in the PCR reaction mixture were 1 X PCR buffer, 200 µM dCTP, dTTP, dGTP, 20 µM dATP, 0.12 µM each primer, 0.25 U of AmpliTaq DNA polymerase (Perkin-Elmer, Foster City, CA) and 0.025 µl [α-^{32}P]ATP (DuPont NEN, Boston, MA, specific activity 3000 Ci/mmol, 10 mCi/ml). A Biomek 1000 or 2000 automated laboratory workstation (Beckman, Fullerton, CA) was used to set up the PCR reactions in 96-well plates. The PCR cycling consisted of 94°C for 3 min followed by 25 cycles of 94°C for 1 min; 55°C for 1 min; and 72°C for 30 sec. A final 72°C extension step was carried out for 5 min. The samples were heat-denatured, mixed with 1 µl loading dye, loaded on 5% sequencing gels and resolved. Gels were exposed to a phosphor screen, scanned using a Phosphoimager (Molecular Dynamics, Sunnyvale, CA), and analyzed quantitatively using ImageQuant software. An allelic imbalance such as loss of

heterozygosity or gene duplication was defined as a 25% or greater difference in the amount of radiolabel incorporation into the PCR products for the individual alleles once normalized to a F1 control spleen DNA sample. Microsatellite instability was identified as any change in allele sizes. All allelic imbalances initially observed were confirmed by repeating the PCR and quantitative analysis at least one time.

RESULTS AND DISCUSSION

Objective 1. Testing of SSR markers over entire genome.

Additional microsatellite markers have been obtained from Research Genetics (from the published map of Jacob *et al.*, 1995), as well as several unpublished ones from the lab of Dr. Howard Jacob, a few from Dr. Ron Wilder (NIH), and some from the literature. Those that are informative have been added to our overall map of the genome and have been tested for linkage to the MCS phenotype. The updated genome map shown in the appendix contains 267 markers compared with the 143-marker map at the time the first annual report was submitted. Besides the *Mcs-1* and *Mcs-2* loci (see Objectives 2 and 3), we found two other regions on chromosome 1 and chromosome 7 that show suggestive linkage (LOD score around 2 to the resistance phenotype. We are currently testing more markers from various sources to clarify this linkage.

In the last year, several markers were added to chromosome 7 and among them, one marker named R1703 showed a LOD score of 2.9 when using tumor number as the quantitative trait. If we use the square root of tumor number rather than tumor

number itself as quantitative trait, the LOD score becomes 3.85, indicating that the second mammary carcinoma suppressor gene (*Mcs-2*) does exist in the area near R1703.

Objective 2. Fine-mapping *Mcs-1* on chromosome 2.

We had experienced difficulties in obtaining more markers for fine-mapping the region of chromosome 2 surrounding the *Mcs-1* locus because of the problems with background contamination. Recently, a chromosome-2-specific library was made successfully and we are isolating new SSR markers from this library for further mapping of *Mcs-1*.

Using markers from this library, as well as from the other sources listed above, we have doubled the number of markers on the chromosome 2 map by adding 27 new markers since the last progress report (see appendix). Of these 27, 12 were from our chromosome-2-specific libraries and one was from an unpublished gene sequence. Twenty-two of the new markers were mapped in the (Cop X WF) F1 X WF backcross, but five were mapped in other crosses (data not shown). Importantly, three markers from our libraries mapped in the previous gap near *Mcs-1* and two of them (CA2.F1 and UW91) are linked to the phenotype. Thus we now have flanking markers linked to *Mcs-1*. Using the square root of the tumor number in the quantitative analysis, markers CA2.F1 and UW91 yielded LOD scores of 4.154 and 2.353, respectively. The LOD for M13, previously the closest marker, is 3.569. The improved LOD score clearly confirms *Mcs-1* as a QTL, and we have decreased the 1 LOD support interval to approximately 12 cM, with the peak LOD being at marker

CA2.F1. Since there is still a 9 cM gap between CA2.F1 and UW91, we are continuing to isolate and map more markers.

As an additional approach to further characterizing the MCS genes, we constructed a F2 mapping panel using Cop and WF rats as F0 strains. This panel consists of 252 animals and we are currently testing the markers that are close to the two MCS gene regions. We expect this mapping panel will give us a better understanding of these two genes.

Objective 3. Fine-mapping *Mcs-2* on chromosome 7.

We generated a chromosome-7-specific library, hybrid-selected for $(CA)_n$ repeats, and analyzed 302 clones from this library. The average insert size was around 600 bp. Among the 302 clones, 139 clones contained $(CA)_n$ repeats as revealed by colony PCR and were then sequenced. Eighty nine clones showed unique sequences, while the others were duplicate clones. Of the 89 sequences, 54 contained enough sequences on both side of the repeats so that 54 PCR primer sets could be designed from the flanking sequences. Among the 54 markers, 12 showed size variations between Cop and WF strains and they were genotyped in the (Cop X WF)F1 X WF backcross. Ten markers mapped back to rat chromosome 7 (see appendix), one to chromosome 9 and one to chromosome 17. Two markers, UW28 and UW35, flank *Mcs-2*. The distance from *Mcs-2* to UW28 was estimated about 4.8 centiMorgens (cM), while to UW35 was more than 17.3 cM. We still need more markers to shorten the distance from the gene to flanking markers.

Some markers that were not polymorphic between Cop and WF were polymorphic between WKy and F344 strains. We typed them along with several

chromosome 7 landmarks in the (WKy X F344)F1 X F344 backcross in order to estimate their relative positions to *Mcs*-2. In this case, we have found a marker named UW25 that is likely to be between *Mcs*-2 and UW28.

In order to reveal the physical location of *Mcs*-2 on rat chromosome 7, we obtained two P1 clones from Genome Systems, Inc. (St. Louis, MO) which contain the sequence of the marker R1703 and used the plasmid DNAs as probes for fluorescence *in situ* hybridization (FISH) analysis of whole rat chromosomes. One clone maps to chromosome 7q13 as expected, confirming the location of *Mcs*-2 on a region close to the centromere of rat chromosome 7. To our surprise the other clone maps to rat chromosome 1q43, with slight cross signals on chromosome 7. What interested us is that the marker R1703 itself was also once mapped to chromosome 1 (personal communication, Research Genetics). Additionally, we found a clone from our chromosome 7 library that shows identical flanking sequences with the rat chromosome 1 satellite sequence D1MCO2 (Gu, L *et al.*, 1996). If these are not coincidences, there might be some kind of unknown relationship between certain regions of these two rat chromosomes. We are characterizing it while we are working on the further mapping of *Mcs*-2.

Although some useful markers have been generated, we were not able to get more markers from this chromosome-7-specific library mainly because:

1. It seems that most of the unique clones from this library have already been identified. At the initial screening stage, most of the clones were unique. However, as more clones were sequenced, fewer clones appeared to be unique. After 200 to 300 clones had been sequenced, we could only get one unique sequence from every 2 or 3 clones, thus we assumed that the library was short of novel unique clones. As there is not a simple method to tell whether a clone is unique prior to sequencing,

and sequencing more and more duplicate clones is costly, we decided to move on to another library.

2. Many clones from this library contained inserts that had repeats at or near the end, therefore we were usually not able to pick up enough flanking sequences for PCR primer design. We assume this may result from the DOP-PCR primer used in making the library. The primer, which has 3' end as TGTGG, might easily anneal to $(CA)_n$ repeat regions during the low annealing temperature DOP-PCR stage and thus amplifies many sequences with $(CA)_n$ repeats at one end.

Due to the two reasons described above, we decided to: 1) make a new chromosome-7-specific library; 2) select for other microsatellite repeats from the remainder of the unselected chromosome-7-specific library. We tried to construct a library from sorted chromosomes by linker-PCR method (VanDevanter *et al.*, 1994) but we did not succeed. At the same time, we were also trying to design new DOP-PCR primers to construct a chromosome-7-specific library from sorted chromosomes. We have obtained some clones from such a library and are currently screening for SSR markers. Hopefully new markers can be obtained from this library soon.

Objective 4. Test for loss of heterozygosity of MCS-linked SSR markers

So far 65 polymorphic markers throughout the rat genome have been tested for loss of heterozygosity in radiation- and DMBA-induced tumors from (WF X Cop)F1 animals. No allelic imbalance was detected in the mapped location of *Mcs-1* on chromosome 2; however, a scan of the genome revealed random allelic imbalance in the radiation-induced tumors. In addition, a non-random LOH on chromosome

1 in the DMBA-induced tumors was documented. The result suggests that loss of *Mcs-1* or *Mcs-2* may not be required in the formation of mammary carcinomas, or that the markers used were not sufficiently close to the *Mcs-1* or *Mcs-2* genes to identify such losses. This experiment will be repeated using additional markers obtained from detailed mapping of the MCS alleles.

CONCLUSIONS

There are few conclusions that can be made as we are continuing the experiments detailed above to further map the rat genome and identify markers which are linked to the mammary carcinoma resistance phenotype in rats. The fact that we are working in the rat means that progress will be slower since large number of markers are not yet available for use as in the mouse and human.

However, besides *Mcs-1* on chromosome 2, we have located a new site on rat chromosome 7 which is linked to the resistance phenotype and represents a second allele, *Mcs-2*. Fine mapping of these two rat mammary carcinoma suppressor genes is in progress. Furthermore, we found two other regions on chromosome 1 and chromosome 7 which may be linked to the resistance phenotype. We are also pursuing these loci to determine whether they are significant.

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APPENDIX

Genetic linkage map of rat genome. This map is constructed in the (Cop X WF)F1 X WF backcross using SSR markers obtained from Research Genetics, the lab of Dr. Howard Jacob (Medical College of Wisconsin), Dr. Ron Wilder (NIH), from the literature, and from the chromosome specific libraries described in the text.

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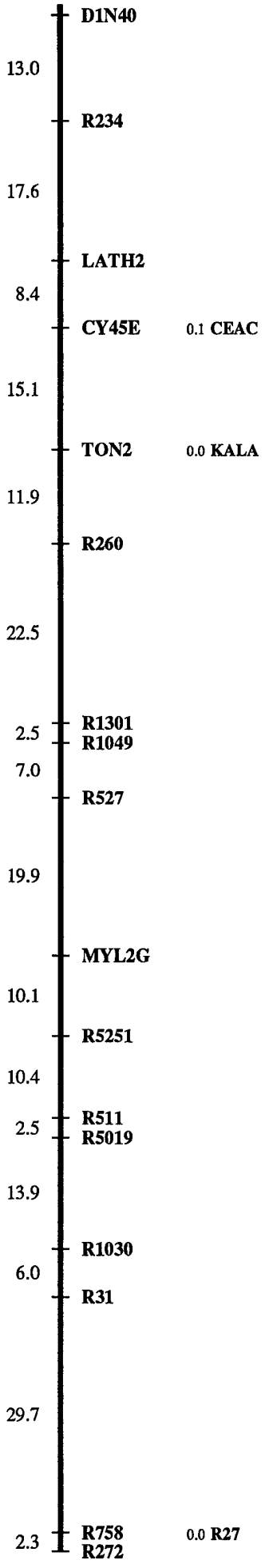

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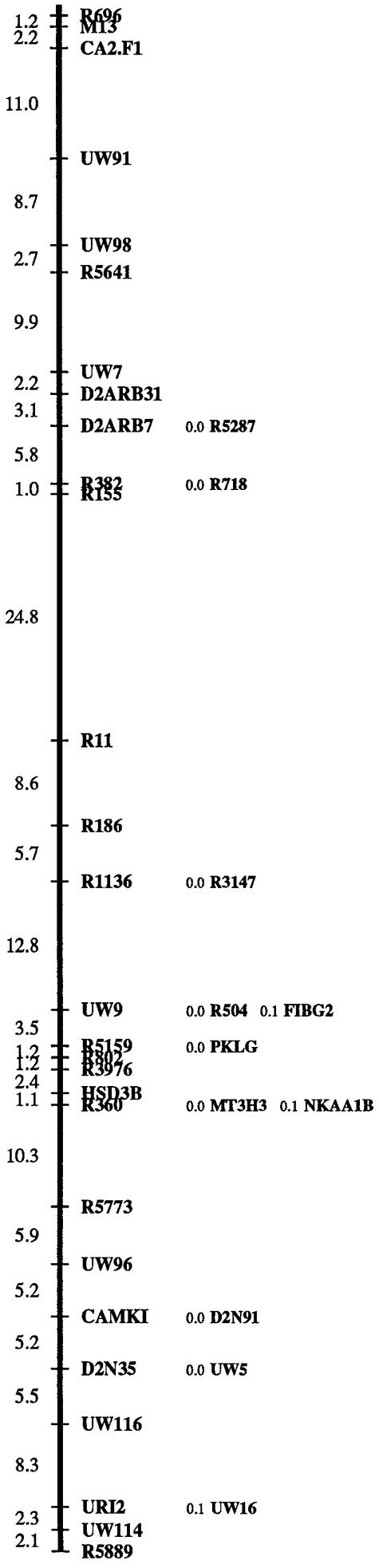

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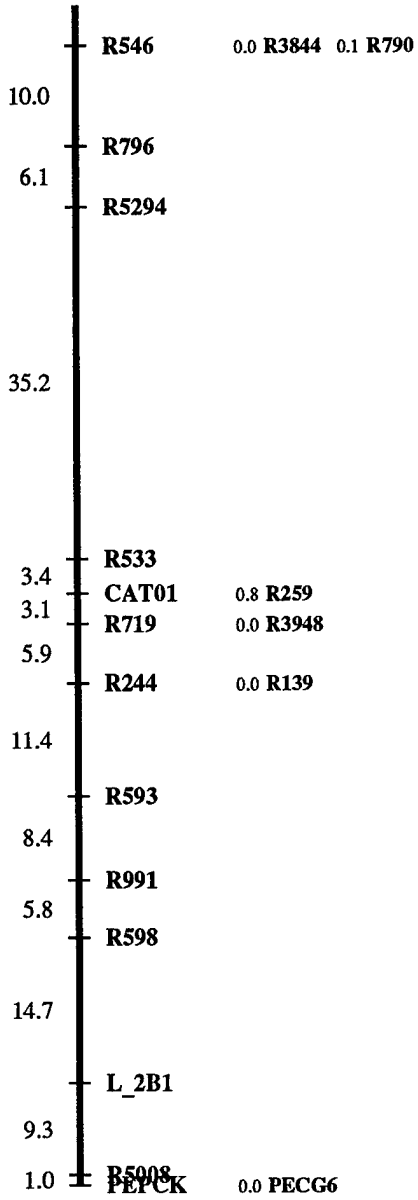

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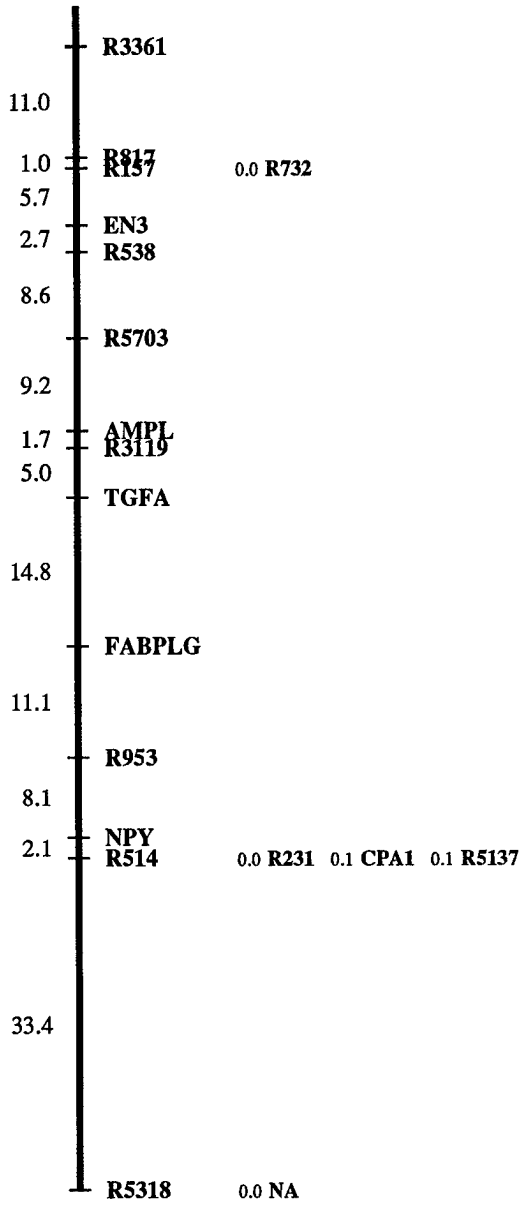

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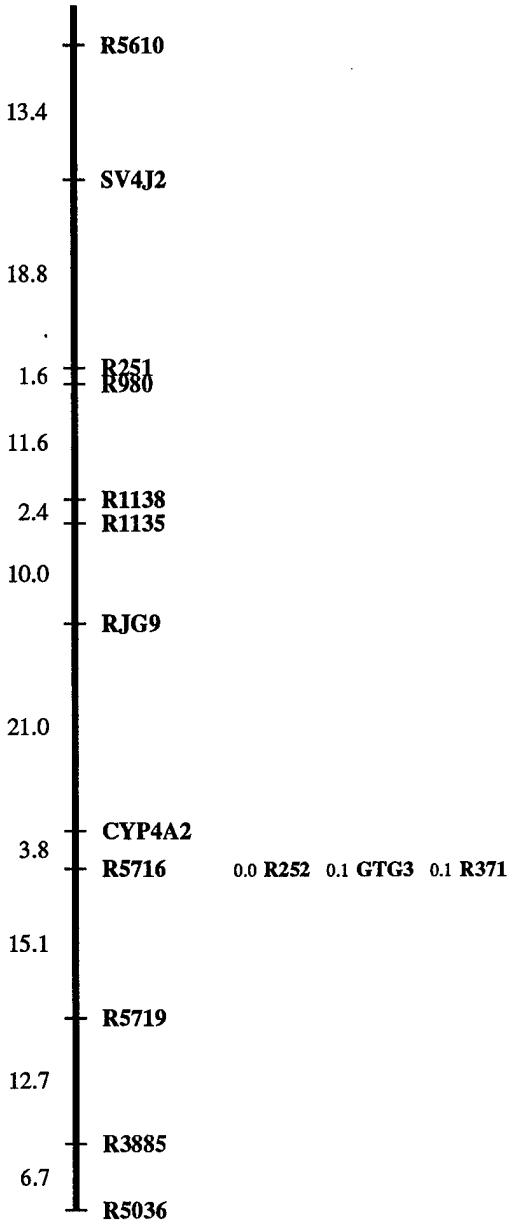

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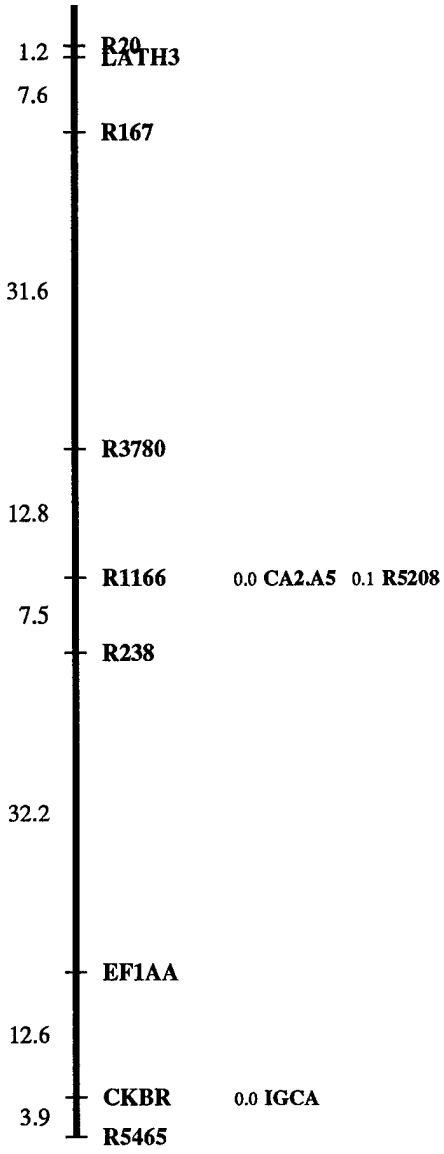

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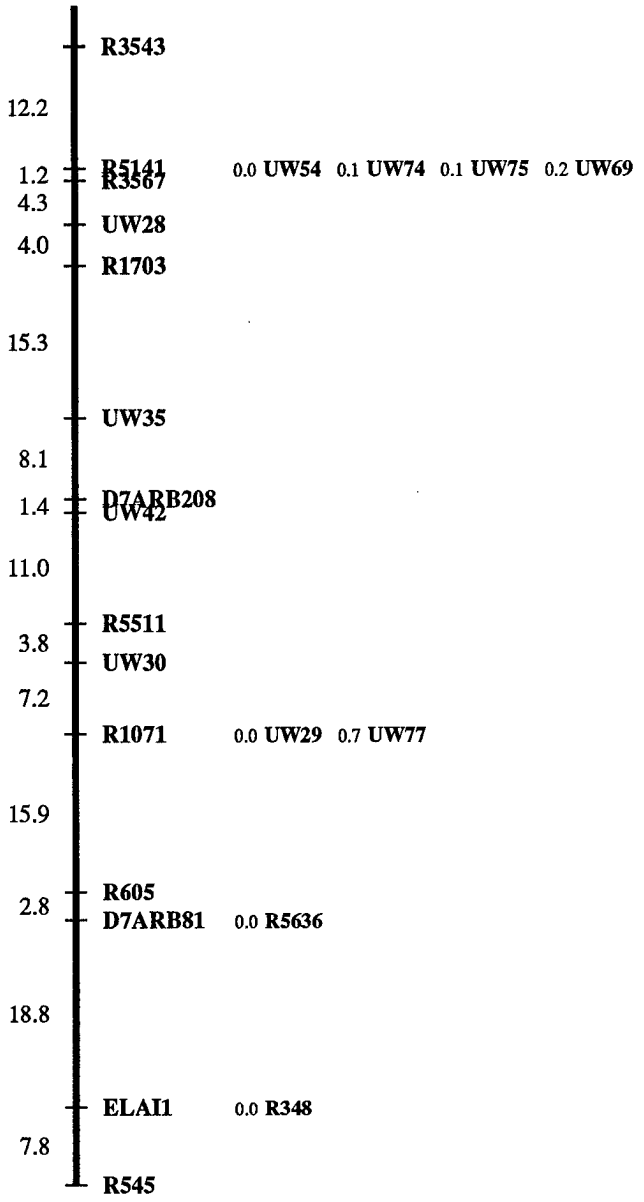

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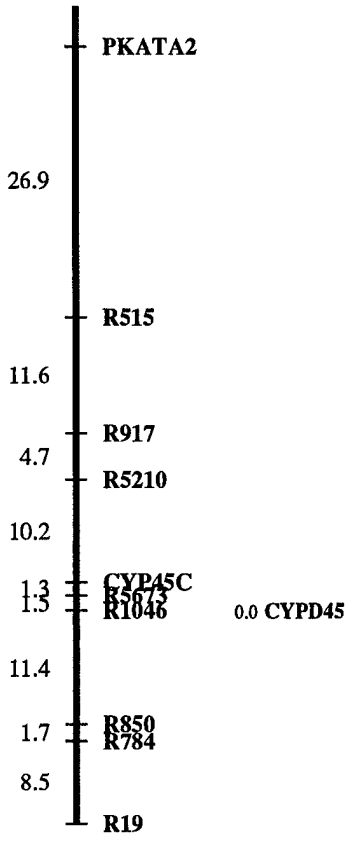

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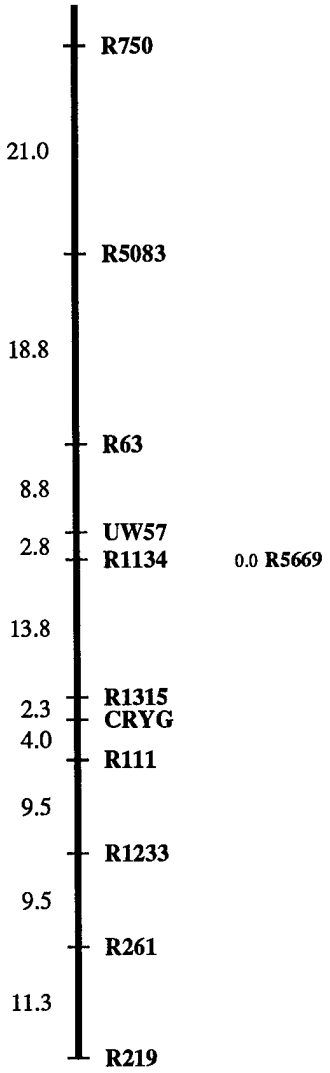

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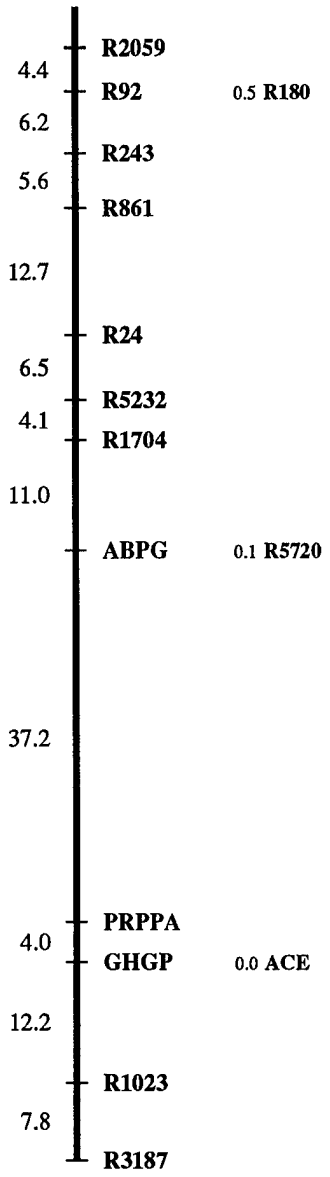

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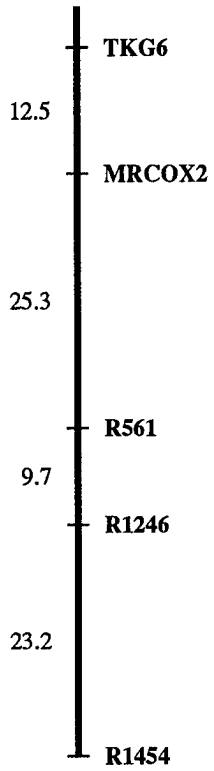

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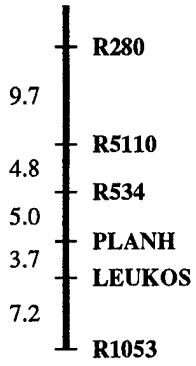

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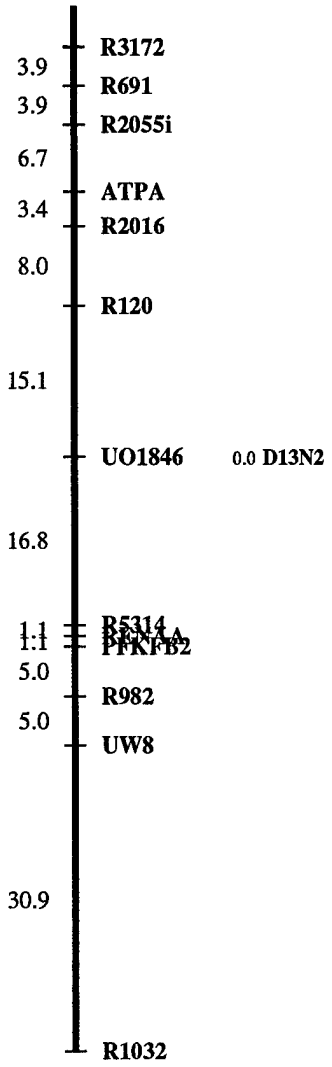

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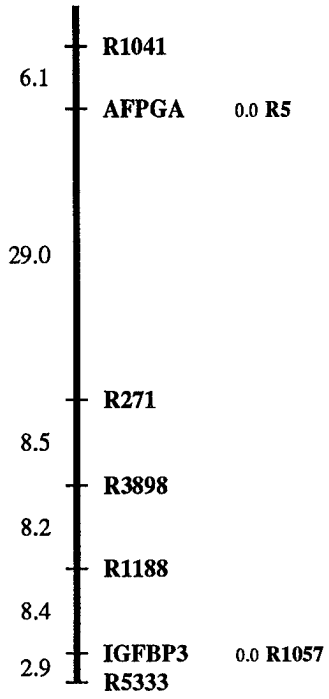

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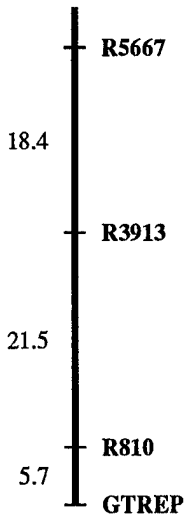

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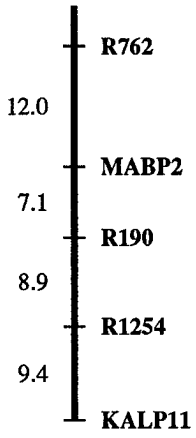

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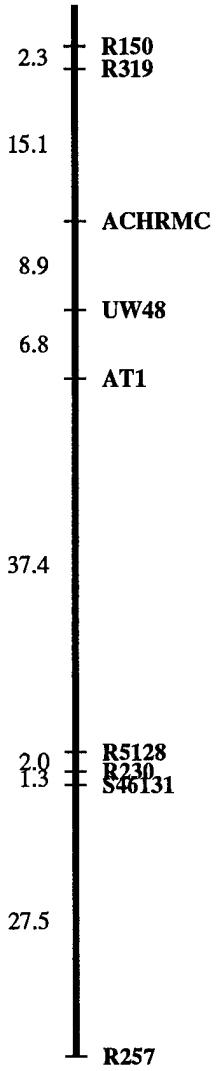

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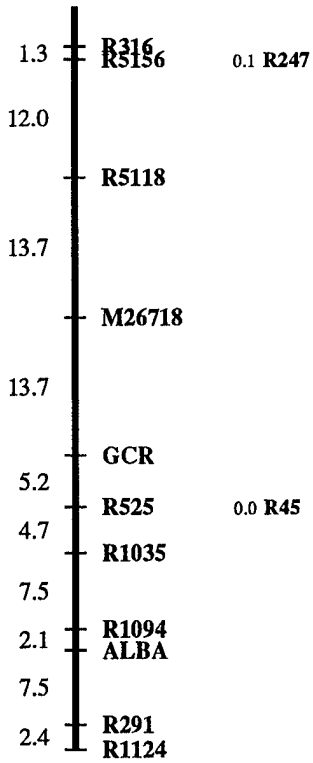

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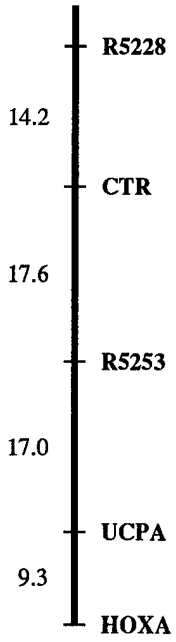

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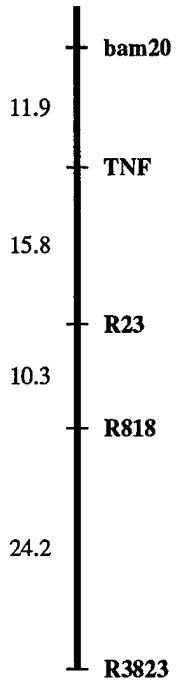

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